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Medical Progress

Platelet Disorders

A Review of Disturbances in Adhesion, Aggregation, and Release Reaction

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WITHIN THE LAST DECADE great progress has been made in our knowledge of the basic cellular mechanisms of blood platelets. The advances helped to clarify the role of platelets in the formation of the hemostatic plug. The tools of investigation readily available to persons interested in the behavior of leukocytes and red blood cells have now been applied to the study of platelets. In addition, new methods, such as aggregometry,[1] platelet factor 3 availability,[2] and kinetic studies of adenosine disphosphate (ADP) and adenosine triphosphate (ATP) release[3-5] have been developed specifically for platelets. A description of all currently used techniques in the study of blood platelets is beyond the limits of this review, but a list of methods with appropriate references is provided in the Appendix.

Platelet Role in Primary Hemostasis: Current Concepts

Normally, platelets circulate freely (partially because of their negative surface potential[6]) in disc form and are carried along separately and are not attracted to the endothelium. After injury of a blood vessel, platelets immediately adhere to exposed collagen[7,8] and other subendothelial fibers.[9,10] Adhesion causes the platelet to swell, changing its shape to a sphere. Approximately 30 to 60 seconds later, adhered platelets rapidly release a large quantity of ADP (and ATP), which results in rapid cohesion of additional platelets. The released ADP can cause release of ADP by other loosely associated platelets.[11] The size of this initial loosely held together mass is most likely related to the number of platelets, to the fluid dynamics at the site of injury, to the amount of ADP released, and to the amount of ADP simultaneously degraded to adenosine monophosphate (AMP) and inosine monophosphate (IMP) by plasma.[12] The specific factors required for platelet aggregation include the following: rapid multiple collisions and the presence of fibrinogen, calcium ions, and ADP. Intrinsic clotting is activated by the effect of ADP on initiation of platelet factor 3 availability and the simultaneous collagen activation of contact factors[13] with the resultant formation of small amounts of thrombin. Thrombin can then induce more platelet clumping as well as render the primary platelet plug impermeable by sealing it with a fibrin mesh. This subject has received critical review.[14]

Classification of Platelet Disorders Based on Morphologic Criteria

The newly developed techniques for study of platelets have been used to study some of the classic bleeding disorders as well as new ones.

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The term *thrombocytopathia,*[15] used previously to describe a bleeding disorder with a disturbance in platelet factor 3 activity, must now be defined more broadly if it is to continue to serve. This is because many of the so-called thrombocytopathias have now been shown to have impaired platelet aggregation in response to ADP and collagen in addition to impaired platelet factor 3 availability. As will be discussed later, disturbances in platelet aggregation exist with or without impaired platelet factor 3 activity; however, an isolated defect in platelet factor 3 availability is unusual when all platelet function tests are employed.

It is difficult to bring order to the classification of platelet disorders because of the confusion in terms, the extensive variety of laboratory methods employed to characterize these disturbances in the past, and the progressive obsolescence of the existent classification resulting from the rapid expansion of the field. The terms *athrombia* and *thrombocytoasthenia* offer no advantages and should be discarded. In addition, recent demonstration of a relationship between size and function of platelets in the normal state[16] (young large platelets aggregate more strongly in response to ADP[17] and adhere more quickly to collagen[18] than old small platelets) provides a useful classificatory criterion for the discussion of platelet disorders. For this reason, platelet disorders will be reviewed according to their association with microthrombocytes, euthrombocytes, and macrothrombocytes (Table 1). Within each class disorders are divided into *inherited* and *acquired* and are presented in order of frequency.

A bleeding disorder is considered associated with microthrombocytes if the percentage of large forms (megathrombocytes, more than 2.5 μ in diameter) is significantly less than the normal percentage of large forms.* In some instances, assessment of volume-density distribution revealed a preponderance of light small platelets consistent with a reduction in the number of large forms. Similarly, conditions in which the percentage of large forms is significantly greater than the normal percentage are classified as associated with macrothrombocytes. All other conditions are considered in the euthrombocyte group, including a few disorders in which platelet size has not been studied.

*Percentage of large forms (more than 2.5 μ in diameter) in normal subjects ranges from 8 to 14 percent (N=37) (personal observations).

TABLE 1.—*Classification of Platelet Disorders Based on Morphologic Criteria*

I. Disorders associated with microthrombocytes (proportion of large forms less than 8 percent)
- A. Inherited disorders
  - 1) Familial adenosine diphosphate release dysfunction
  - 2) Wiskott-Aldrich syndrome
  - 3) Hereditary hypogranular thrombopathic thrombocytopenia
- B. Acquired disorders
  - 1) Uremia
  - 2) Platelet defects in refractory anemia in preleukemic state

II. Disorders associated with euthrombocytes (proportion of large forms 8 to 14 percent)
- A. Inherited disorders
  - 1) Essential thrombasthenia
    - (a) Resulting from enzymopathy
    - (b) Resulting qualitative disturbance of membrane and membrane-bound thrombasthenia with decrease in platelet function
  - 2) Familial adenosine diphosphate release dysfunction
  - 3) Albinism associated with adenosine diphosphate release dysfunction
  - 4) Congenital afibrinogenemia
  - 5) Glycogen storage disease
  - 6) Mongolism (trisomy 21)
- B. Acquired disorders
  - 1) Drug-induced platelet dysfunction
  - 2) Cirrhosis
  - 3) Nonfamilial adenosine diphosphate release dysfunction
  - 4) Essential thrombocythemia
  - 5) Polycythemia vera
  - 6) Macroglobulinemia

III. Disorders associated with macrothrombocytes (proportion of large forms greater than 14 percent)
- A. Inherited disorders
  - 1) Macrothrombopathia
  - 2) May-Hegglin anomaly
  - 3) Heritable disorders of connective tissue
  - 4) Gray platelet syndrome

Symptomatology of Bleeding Disorders Owing to Platelet Dysfunction

In general, the clinical manifestations of an intrinsic platelet defect are usually skin purpura after minor trauma, dependent petechiae, and mucocutaneous bleeding. In addition, epistaxis, rectal bleeding, and unexplained hematuria sometimes occur. Hemarthrosis is distinctly unusual even in the most severe disorder, essential thrombasthenia. The bleeding tendency varies from time to time in each patient and there may be symptom-free periods. Abnormal bleeding during and after surgical operation is unpredictable.

Disturbances in the platelet number (for example, in essential thrombocytosis or thrombocytopenia) may also occur in addition to intrinsic defects. In patients with thrombocytopenia, spontaneous bleeding usually does not occur until the platelet count falls below 30,000 per cu mm even though impairment of clot retraction often occurs at the 100,000 per cu mm level. When bleeding does occur with platelet counts between 50,000 and 100,000 per cu mm, excluding a surgical lesion, a qualitative defect in the platelet is probably present. Drugs that interfere with platelet function, such as aspirin, should always be avoided since there are adequate substitutes available.[19,20] In the inherited conditions platelet transfusions have been temporarily used to arrest bleeding and to prevent bleeding during surgical operation. In the acquired disorder, platelet transfusions are not effective since the underlying condition must be treated.

Disorders Associated with Microthrombocytes*

Inherited Disorders

Familial ADP *release dysfunction.* This mild familial bleeding disorder is characterized by the presence of small platelets, a prolonged bleeding time (or profound prolongation of the bleeding time after aspirin ingestion), normal or impaired platelet factor 3 availability, normal whole blood clot retraction, and defective platelet aggregation in response to collagen suspension, epinephrine, low molar ADP, and dilute thrombin.[21-24] The mode of inheritance is not clear, but appears to be autosomal-recessive in two families[21,22] and autosomal-dominant in one. The incidence of this disorder has not been determined as yet. The aggregation studies were carried out at 37°C in citrated platelet-rich plasma (CPRP). Aggregation does not occur in response to collagen unless the concentration used is much higher than that used to evoke a normal response in control CPRP. The first wave of epinephrine-induced aggregation is normal or blunted and the second wave is absent. Rapid disaggregation occurs after addition of low molar ADP to the test system. Platelet adhesion to collagen fibrils, measured by phase microscopy, is normal but adhesiveness, measured by the glass bead method, is abnormal. The percentage of large forms is significantly reduced and the disturbance appears to be caused by a "block" in the release of ADP from the platelet after exposure to collagen and epinephrine. Intraplatelet nucleotide levels are reduced[21,22] and depletion of the "nonmetabolic" pool of ADP and ATP appears to account for the disturbance.[24] Platelet survival studies have not been carried out, as far as is known. Transfusion of platelets temporarily corrects the hemostatic disorder.[22]

It is possible that small platelets with reduced ADP levels are manifestations of several different disturbances in intraplatelet structure and function. Ineffective incorporation of inorganic phosphate may result from a disturbance in the platelet membrane or a defect in energy production or utilization. The effect would be a reduction in releasable ADP during induction of the release reaction. A reduction in the ability of the cell to maintain levels of high energy nucleotides may result in loss of cell mass. More investigations are needed to clarify the mechanisms operative in this condition.

Wiskott-Aldrich syndrome. This disorder is transmitted as a sex-linked recessive trait and manifests clinically in infancy with eczema and recurrent infections secondary to deficiencies in both humoral and cellular immune responses. In addition, mild to severe thrombocytopenia exists and about 24 percent of the patients have severe hemorrhagic diathesis not necessarily related to the severity of the thrombocytopenia. Platelets in peripheral blood smears are unusually small with a mean diameter of less than 2μ.[25] Examination of platelets reveals a lack of granulations by light microscopy and a decrease in organelles by electron microscopy.[26] A reduction in platelet electrophoretic mobility normally observed after exposure of platelets to collagen and ADP is not observed.[26] Platelet adhesiveness and platelet factor 3 availability are impaired. Aggregation in response to ADP, collagen, and epinephrine is decidedly impaired.[27] The heterozygous carrier can also be detected by such disturbances in platelet aggregation. A lack of stimulation of intraplatelet citric acid cycle activity after exposure to epinephrine or polystyrene-latex particles is observed in both the homozygous patients and the heterozygous carrier.[27] The survival time of autologous platelets is considerably reduced. It is likely that the intracorpuscular disturbance causes rapid platelet senescence and sequestra-

*Proportion of large forms less than 8 percent.

tion by the reticuloendothelial system. One of the two patients with this disorder studied in our laboratory does not have the characteristic disturbances in platelet aggregation, but the immunologic deficiency, thrombocytopenia, and microthrombocytes are present. It is likely that several distinct intracorpuscular disturbances exist.

Hereditary hypogranular thrombopathic thrombocytopenia. In 1958, a Swiss family with a bleeding disorder characterized by hypogranular platelets and thrombocytopenia was reported.[28] The disturbance is transmitted by an autosomally dominant gene and occurs with the allele for blood group O. Ultrastructural studies of the thrombocytes and megakaryocytes of some of these patients show a severe disturbance in dense granules in both platelets and megakaryocytes.[29] Platelet factor 3 availability and aggregation with collagen suspensions are defective in these patients. The content of sialic acid in the platelet membrane is low and the number of large platelets is considerably reduced.[29]

Acquired Disorders

Uremia. Although vascular and plasma factors have been proposed in the past to explain the cause of uremic bleeding, it has become obvious that most of the bleeding abnormalities are related to acquired functional platelet defects. Patients with renal failure and a bleeding tendency usually have prolonged bleeding times, impaired platelet adhesiveness,[30,31] and defective platelet aggregation *in vitro* in response to ADP, epinephrine, and dilute thrombin;[32] however, impaired aggregation in response to collagen is rare (personal observations). Activation of platelet factor 3[33,34] and prothrombin consumption are impaired in 40 percent of patients. In our studies of 11 patients undergoing chronic hemodialysis the mean platelet size was small (less than 2μ) and the percentage of large forms considerably reduced. This most likely reflects reduction in megakaryocytopoiesis or loss of cell mass owing to the effect of a uremic toxic agent on platelet metabolism. Platelets from uremic patients have normal phospholipid[35] and adenine nucleotide concentrations,[36] but the concentration and uptake of serotonin are impaired.[37] The platelet defect in patients with uremia can be produced in the normal subject by infusion or oral administration of large amounts of urea.[38] The defects in platelet aggregation and platelet factor 3 availability can also be produced *in vitro* by incubating cell-free uremic plasma with normal platelets.[39] Furthermore, these platelet defects are reversible by hemodialysis over a period of 12 to 24 hours.

The specific toxic agent responsible for these disturbances in platelet function is still debated, but the most likely agents are urea, guanidinosuccinic acid,[40] and phenol and phenolic acids.[41,42] One group of investigators observed that only guanidinosuccinic acid is present in uremic plasma in concentrations sufficient to cause an identical platelet disturbance *in vitro*.[40]

Platelet defects in refractory anemia in preleukemic state. A platelet disorder that accompanies the refractory anemia of the preleukemic condition has been observed.[43,44] In these patients the low grade anemia is associated with leukocytosis, low levels of leukocyte alkaline phosphatase, and elevated platelet counts. The numbers of myeloblasts and promyelocytes in bone marrow are increased. In many respects these patients resemble patients with chronic myelocytic leukemia before blastic crisis. The bone marrow contains numerous nonlobulated megakaryocytes and the peripheral blood contains only small-sized platelets. Platelet aggregation is defective and the incorporation of ^{14}C-adenosine is impaired. Platelet survival is decreased.[44]

Disorders Associated with Euthrombocytes*

Inherited Disorders

Essential thrombasthenia. This familial autosomal-recessive bleeding disorder is characterized by a normal platelet count, prolonged bleeding time, decreased or absent clot retraction, and complete absence of platelet aggregation in response to ADP, measured by aggregometer.[45-47] More than 100 cases have been reported.[47-49] The carrier state may be associated with impaired clot retraction,[50] although results of other tests of platelet function are within normal limits. On the peripheral blood smear the platelets appear isolated and do not spread normally on a glass slide. Addition of ADP to CPRP results in an occasional small clump of platelets not sufficient to be recorded as a change in optical density by the aggregometer. The platelets adhere normally to

*Proportion of large forms 8 to 14 percent.

subendothelial connective tissue. The nucleotide and 5-hydroxytryptamine concentrations in platelets are within normal limits. Platelet factors 3 and 4 availability is impaired.[46] Platelet aggregation can be induced only by bovine fibrinogen or antiplatelet antisera.[46] Release of nucleotides, serotonin, and acid phosphatase is impaired in response to ADP and epinephrine but is normal in response to collagen and thrombin.[51] In contrast to thrombasthenia, only trace amounts of nucleotides and serotonin are released after collagen or thrombin in primary ADP release dysfunction. The life span of autologous platelets in thrombasthenia is normal.

Several investigators have reported disturbances in carbohydrate metabolism with decreased levels of ATP. In addition to a deficiency of glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase,[52] a defect in glutathione reductase has been observed.[53] Other investigators have found a decrease in platelet fibrinogen without a disturbance in metabolism.[8,54] A recent hypothesis is that the interaction of fibrinogen with platelet surface thrombasthenin might be impaired.[51] The only effective therapy appears to be transfusion of platelet concentrates or PRP; this was employed successfully in the delivery of a healthy baby from a thrombasthenic mother.[55]

Familial ADP *release dysfunction.* A family with three affected members with a platelet defect similar to that described for the disorder associated with microthrombocytes but transmitted as autosomal-dominant with variable transmission through two generations has been reported.[56] However, in these patients platelet adhesion to collagen fibrils is impaired. This platelet disturbance is distinguished from that induced by aspirin by a more rapid disaggregation after addition of low molar ADP to CPRP, with reduced platelet adhesiveness, and a frequently prolonged bleeding time. In addition, the first wave of epinephrine-induced aggregation is blunted. Aspirin does not cause any of these disturbances in aggregation.[57]

Clinically, manifestations include excessive bruising, petechiae, unexplained hematuria, and epistaxis. In some instances platelet transfusions have been helpful during bleeding episodes. It is impossible to predict if hemorrhagic complications will occur during surgical operation.

Albinism associated with ADP *release dysfunction.* A possible association between albinism and a bleeding disorder has been reported many times.[58,59] In 1967, 13 patients with a hemorrhagic diathesis characterized by normal platelet count, prolonged bleeding time, and defective platelet aggregation after addition of collagen to PRP were reported. Two patients were unrelated women with albinism. In a third patient with total albinism and a mild bleeding disorder, platelet aggregation was impaired after addition of not only collagen but also epinephrine and dilute thrombin.[60] The platelet disturbance appears to be a block in the release of ADP similar to that in other recently described platelet functional disorders.[24-27] In some instances intracellular concentrations of ADP and ATP were subnormal.

Congenital afibrinogenemia. This is a rare disorder transmitted by an autosomal recessive gene. Just over 20 cases have been reported.[60a] Patients with congenital absence of fibrinogen usually have a prolonged bleeding time and decreased glass bead adhesiveness.[61-63] Since washed platelets aggregated poorly *in vitro* when fibrinogen is not added,[64] it is not surprising that platelet aggregation in these patients is impaired. Specifically, the defect occurs in response to low molar ADP, epinephrine, and collagen but only in CPRP; higher strengths of ADP and collagen induce normal aggregation. No platelet aggregation disturbance is present *in vitro* when heparinized PRP is used.[65,66] The reason for this discrepancy is unclear. Impaired release of ADP has been observed in response to kaolin and epinephrine but not to connective tissue or thrombin.[66] The distribution of platelet size and the nucleotide concentration appear uneffected.

Plasma transfusions correct the abnormal bleeding time and platelet adhesiveness. Addition of 10 to 20 mg per 100 ml of fibrinogen to CPRP *in vitro* corrects most of the platelet aggregation defects.[66] Since the platelets adhere normally to subendothelial collagen the exact way in which fibrinogen aids in the arrest of bleeding remains unknown. More than likely it is responsible for bridging one platelet to another during the aggregation phenomenon.

Glycogen storage disease. A bleeding tendency has been reported in patients with hepatomegalic forms of glycogen storage disease Types I, III, and VI.[67] Prolonged bleeding time, de-

creased platelet adhesiveness, and impaired platelet factor 3 availability have been observed in Type I of the disease.[68,69] The defect is believed to be related to the large excess of stored glycogen in the platelets. Adequate studies have not been carried out in Types III and VI. Platelet aggregation and nucleotide concentration and release have not been studied.

Mongolism (trisomy 21). In this disorder the platelet concentration of serotonin is low and the uptake of serotonin is impaired.[70,71] Platelet function defects may be present but sufficient data have not been obtained.

Acquired Disorders

Drug-induced platelet dysfunction. Although more than 50 drugs have been reported to alter platelet function[72] only the most widely studied compound, acetylsalicylic acid, will be discussed.

It has been known for many years that ingestion of aspirin aggravates bleeding, and patients with hemophilia have avoided this drug when possible. Several investigators have shown that aspirin ingestion prolongs the bleeding time.[73-76] Quick[77] popularized this finding and has been justifiably critical of the indiscriminate use of this drug in patients with a bleeding or clotting disorder. Aspirin affects platelet function by inhibiting the release of ADP[78] from platelets exposed to release inducers, such as epinephrine,[79] ADP[11] and dilute collagen,[75] but it does not inhibit release of ADP induced by concentrated collagen suspensions or thrombin. The second wave of epinephrine-induced platelet aggregation is blocked.[79] Ingestion of as little as 150 mg of aspirin can produce these effects, which can last for four to seven days even though the half-life of intravenously administered aspirin is 13 to 19 minutes.[80] Aspirin has no demonstrable effect on platelet nucleotide levels, surface charge, or rate of ADP conversion in plasma.[75] High concentrations of aspirin inhibit uptake of glucose[81] and ascorbic acid[82] and production of lactate.[81] It has been suggested[83] that aspirin interferes with early reactions in the biosynthesis of nicotinic acid adenine dinucleotide in platelets. Aspirin also blocks degradation of radioactive ATP to IMP after previous incubation and incorporation of inorganic ^{32}P into platelets.[84] This block in ATP degradation may indicate interference with the energy necessary for the release reaction.[24]

Sodium salicylate does not affect platelet function or prolong the bleeding time.[75] However, acetic anhydride, another acetylating agent, produces a defect identical to that produced by aspirin: $1\text{-}^{14}C$-acetylsalicylic acid effects acetylation of human platelet membranes and granules.[85] It is most probable that the acetyl radical is the active moiety producing all the platelet defects.

Initially the investigatory emphasis was on the potentiation of bleeding states by aspirin; this problem is solved by avoidance of the drug. The investigation of the antithrombotic potential of agents that induce a mild platelet defect and may thereby inhibit the genesis or propagation of arterial and venous thrombi is more important and urgent.

Cirrhosis. The hemorrhagic tendency in patients with cirrhosis of the liver may be aggravated by thrombocytopenia, low procoagulant levels, abnormal elevation of fibrin-split products, and defective fibrin polymerization. Platelet adhesiveness is elevated[86] in some patients and reduced in others.[87] Platelet procoagulant activity is defective in a significant number of patients, but this does not appear related to clinical bleeding. In one study, adenosine diphosphate-induced and thrombin-induced platelet aggregation was impaired in 9 of 20 patients with cirrhosis.[88] However, the platelet defect was observed only in association with a prolonged thrombin time,[88] which is consistent with accelerated fibrinolysis and commonly found in advanced liver disease. The increased levels of circulating fibrin-split products not only act as an antithrombin in prolonging the thrombin time, but also interferes with platelet aggregation.[89]

Nonfamilial ADP release dysfunction. This isolated bleeding disorder is characterized by normal platelet count, prolonged bleeding time, decreased platelet adhesiveness to glass, and defective platelet aggregation in response to collagen, epinephrine, and low molar ADP.[57,90-95] The patients suffer from excessive bruising; the degree of bleeding during surgery is unpredictable. In many respects this disorder is similar to the inherited platelet dysfunction associated with microthrombocytes, although no inheritance pattern has been detected. Levels of all coagulation factors are within normal limits. Clinic-

ally, this disorder is similar to von Willebrand's disease; only platelet aggregation studies differentiate the two.[96] The distinction between this disorder and that induced by aspirin ingestion is sometimes troublesome.

Essential thrombocythemia. The paradoxical association of a hemorrhagic tendency in patients with a myeloproliferative disorder with increased numbers of platelets has often been attributed to a platelet defect. In three patients with this disorder, platelet aggregation in CPRP was absent in response to epinephrine and reduced after addition of ADP.[97] The platelets showed normal aggregation to collagen. Uptake of ^{14}C-labeled serotonin was diminished. After intravenous administration of 4 mCi ^{32}P to a patient, the platelet count dropped from 5 million to 500,000 per cu mm.[97] Platelet aggregation in response to epinephrine and ADP gradually returned to normal values.[97] It appears that the platelet disturbance is a reflection of the disease state and not just of the elevated platelet count.

Polycythemia vera. In five of our six patients with polycythemia vera who were untreated, platelet aggregation in response to epinephrine was totally absent (personal observations). The ADP-induced platelet aggregation was defective in only two of the six patients and was not related to the platelet count. The significance of these abnormalities *in vivo* is unclear.

Macroglobulinemia. In this acquired platelet disorder an interaction between the platelet membrane and macroglobulins occurs.[98] The bleeding disorder is readily corrected by plasmapheresis. Impairment of platelet factor 3 activity, prothrombin consumption, and platelet aggregation *in vitro* is corrected with removal of the macroglobulins by platelet washing.[98] The physicochemical adsorption of macroglobulins is believed responsible for the platelet dysfunction. When a patient's platelets are incubated with specific rabbit antihuman macroglobulin serum, the platelet defect is abolished. Monomers of macroglobulin do not produce the defect.[99] Incubation of normal platelets with macroglobulins can induce the platelet dysfunction.[98] In some cases of multiple myeloma, protein aggregates may form and induce a similar disturbance of platelet function. More than likely the infusion of dextran has similar effects on platelet function.

Disorders Associated with Macrothrombocytes*

Inherited Disorders

Macrothrombopathia. This familial disorder of platelet function associated with large platelets, often the size of lymphocytes, was first described by Bernard and Soulier in 1948.[100] Two dozen subsequent reports have described a similar bleeding disorder with abnormal prothrombin consumption, platelet factor 3 availability, and variable defects in platelet aggregation.[101-108] Some patients have moderate thrombocytopenia with a shortened platelet life span. Morphologic abnormalities have been noted in megakaryocytes as well as in the platelets, and correction of the thrombocytopenia by splenectomy is usually unsuccessful. The bleeding tendency appears unrelated to the thrombocytopenia. Hypogranular instead of hypergranular platelets were reported in some cases in which "agglutination" was defective, bleeding time was prolonged, and clot retraction was normal,[101] whereas in eight family members with defective prothrombin consumption and prolonged bleeding time the platelets were abnormally large and granular.[108] The mode of inheritance in this family was autosomal-dominant. In a recent investigation employing newer methods, ADP-induced aggregation was impaired as well as platelet factor 3 activity.[102,104] In addition, in another family platelet adhesiveness was decreased and total phospholipid content was increased although platelet aggregation with ADP and thrombin determined by visual means was normal.[107] The variability in the degree of platelet dysfunction is further emphasized by the results obtained in two other families.[105,109] In one family with eight affected members in three generations, ADP-induced aggregation was normal in all eight, even though electron microscopic studies revealed a low content of alpha-granules in platelets of two patients.[109] In the other family, considered to have a platelet membrane defect, electrophoretic mobility of patients' platelets was abnormal in response to ADP.[105]

Thrombocytopenia associated with large platelets has also been noted in three patients with Alport's syndrome.[106] In these patients, platelet granules were distributed unevenly. Platelet aggregation in response to dilute ADP, collagen and

*Proportion of large forms greater than 14 percent.

epinephrine was impaired, and platelet adhesiveness to glass beads was absent. Kaolin-induced factor 3 availability was diminished. Release of ADP and ATP from platelets in CPRP after exposure to collagen was considerably reduced. Except for the large size of the platelets, ADP release dysfunction in these patients appears to be similar to the platelet ADP release dysfunction classified under euthrombocytes. Furthermore, the ATP content in the platelets dropped quickly during incubation at 37°C, indicating possible impairment of glycolysis.

May-Hegglin anomaly. This is a familial disorder characterized by appearance of leukocyte inclusion bodies (Döhle bodies) and giant platelets on the peripheral blood smear.[110,111] Because the patients are often asymptomatic, the condition may go unnoticed. Thrombocytopenia is present in some patients. In many instances the morphologic disturbance of the platelets is not associated with abnormalities in function.[112] Platelet factor 3 availability is moderately impaired, and bleeding time and platelet adhesiveness and aggregation are normal.[113]

In one family with this disorder no hemorrhagic abnormalities were present despite the mild thrombocytopenia.[112] When Hegglin's original patient was restudied[112] using newer methods, platelet aggregation in response to thrombin was impaired, platelet factor 3 activity was abnormal, and clot retraction was poor.

Heritable disorders of connective tissue. A spectrum of platelet and coagulation disorders was studied in 31 families with heritable disorders of connective tissue.[114] Large platelets were common in most of the patients, but defects in platelet aggregation were inconsistent; defects were present in two of 16 patients with osteogenesis imperfecta, in two of three patients with Ehlers-Danlos disease, in two of five patients with Hurler's disease, and in two of eight patients with Marfan's syndrome. Whole blood clot retraction is sometimes impaired, particularly in patients with Marfan's syndrome.

Gray platelet syndrome. In the only case reported,[115] an 11-year-old boy with recurrent petechial rashes, pronounced bruising and variable degrees of thrombocytopenia, the platelets were large and "of a peculiar gray color." Almost all of the platelets lacked granules and the megakaryocytes also lacked granules. Curiously, the bleeding tendency disappeared after the platelet count rose in response to corticosteroid treatment and splenectomy; however, the morphologic abnormalities persisted. This unusual case underscores the value of careful morphologic observation of platelets in the peripheral blood smear.

Closing Comments

It is now possible to uncover disturbances in platelet aggregation in patients plagued with mucocutaneous bleeding and excessive bruising. These patients had previously been grouped under the heading "pseudohemophilia A" or "bleeding disorder, cause unknown." The incidence of these mild bleeding disorders is probably much higher than was previously appreciated. Patients may at times have a normal bleeding time; however, a profound prolongation of the bleeding time can be observed two hours after aspirin ingestion if a defect exists. Often, careful examination of the peripheral blood smear will reveal a preponderance of large or small forms. In rare instances degranulated forms are noted.

Results of standard tests of bleeding such as platelet count, clot retraction, and prothrombin consumption are often within normal limits. However, the ability of the platelets to aggregate in an *in vitro* test system (aggregometry) is often severely disturbed. Also, activation of platelet factor 3 by ADP, collagen or kaolin may be defective.

Most of the platelet disorders described herein have been investigated intensely by these newer methods. A word of caution is advisable. Since a similar defect can be induced by any number of commonly used drugs, it is reasonable to withhold a diagnosis until the constancy of the defect is determined. The disturbance in platelet aggregation must be found on three successive occasions, during which control specimens are also tested, after abstention from all drugs for seven days. If these conditions are met, a diagnosis can be made. The family should then be carefully studied even when there are no symptoms of bleeding.

The detection of platelet function disturbances is likely to increase. Present attempts to define the subcellular mechanism operative in these conditions should provide a more cohesive picture of the entire spectrum of hemostatic disorders.

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APPENDIX

Techniques Used in the Study of Platelets

- Platelet size distribution
  - Particle size plotter (volume) (1)
  - Micrometric ocular piece (2)
- Megakaryocyte reserve and thrombopoietic potential (3,4)
- Life span utilizing diisopropylfluorophosphate^{32} or chromium^{51} (5,6)
- Cell populations, separation by:
  - Osmotic resistance (7)
  - Differential centrifugation in inert medium (8)
  - Noncontinuous sucrose gradient ultra-centrifugation (9)
- Aggregation with collagen, adenosine diphosphate, epinephrine, thrombin, antigen-antibody complexes (10,11)
  - In whole blood (12)
  - In platelet-rich plasma (10,11,13)
- Adhesion
  - To glass, collagen, and other foreign surfaces (14-18)
- Clot retraction
  - Whole blood (19)
  - Platelet-rich plasma (20)
- Release reaction (21-23)
  - Nucleotide studies (24)
  - Direct quantitation (25)
  - Uptake, localization and release utilizing C^{14} labelled compounds, including serotonin (21-24)

Adenosine diphosphate release, storage, degradation in plasma (26)

Column and thin layer chromatography for mapping intracellular nucleotides (27,28)

Release of potassium, calcium, acid phosphatase, β-glucuronidase (22)

Uptake of glucose, ascorbic acid, amino acids (29,30)

Cellular metabolism

Glycolysis (31)

Citric acid cycle, citric acid metabolism (32, 33)

Oxygen consumption (34)

Incorporation of acetate; synthesis of fatty acids (35)

Protein synthesis (9,36)

Platelet thromboplastic activity—so-called platelet factor 3 (37)

Platelet antiheparin activity (38)

Electrophoresis in response to epinephrine, collagen, adenosine diphosphate (39,40)

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